Protein Kinase C Inhibitors

Protein kinase C (PKC) consists of a family of closely related enzymes that function as serine/threonine kinases. Protein kinase C plays an important role in cell-cell signaling, gene expression, and in the control of cell differentiation and growth. At present, there are currently at least ten known isozymes of PKC that differ in their tissue distribution, enzymatic specificity, and regulation. Nishizuka Y. Annu. Rev. Biochem. 58: 31-44 (1989); Nishizuka Y. Science 258: 607-614 (1992).

Protein kinase C isozymes are single polypeptide chains ranging from 592 to 737 amino acids in length. The isozymes contain a regulatory domain and a catalytic domain connected by a linker peptide. The regulatory and catalytic domains can be further subdivided into constant and variable regions. The catalytic domain of protein kinase C is very similar to that seen in other protein kinases while the regulatory domain is unique to the PKC isozymes. The PKC isozymes demonstrate between 40-80% homology at the amino acid level among the group. However, the homology of a single isozyme between different species is generally greater than 97%.

Protein kinase C is a membrane-associated enzyme that is allosterically regulated by a number of factors, including membrane phospholipids, calcium, and certain membrane lipids such as diacylglycerols that are liberated in response to the activities of phospholipases. Bell, R.M. and Burns, D.J., J. Biol. Chem. 266: 4661-4664 (1991); Nishizuka, Y. Science 258: 607-614 (1992). The protein kinase C isozymes, alpha, beta-1, beta-2 and gamma, require membrane phospholipid, calcium and diacylglycerol/phorbol esters for full activation. The delta, epsilon, eta, and theta forms of PKC are calcium-independent in their mode of activation. The zeta and lambda forms of PKC are independent of both calcium and diacylglycerol and are believed to require only membrane phospholipid for their activation.

Only one or two of the protein kinase C isozymes may be involved in a given disease state. For example, the elevated blood glucose levels found in diabetes lead to an isozyme-specific elevation of the beta-2 isozyme in vascular tissues. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). A diabetes-linked elevation of the beta isozyme in human platelets has been correlated with their altered response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl. 1) 97A (1993). The human vitamin D receptor has been shown to be selectively phosphorylated by protein kinase C beta. This phosphorylation has been linked to alterations in the functioning of the receptor. Hsieh et al., Proc. Natl. Acad. Sci. USA 88: 9315-9319 (1991); Hsieh et al., J. Biol. Chem. 268: 15118-15126 (1993). In addition, recent work has shown that the beta-2 isozyme is responsible for erythroleukemia cell proliferation while the alpha isozyme is involved in megakaryocyte differentiation in these same cells. Murray et al., J. Biol. Chem. 268: 15847-15853 (1993).

The ubiquitous nature of the protein kinase C isozymes and their important roles in physiology provide incentives to produce highly selective PKC inhibitors. Given the evidence demonstrating linkage of certain isozymes to disease states, it is reasonable to assume that inhibitory compounds that are selective to one or two protein kinase C isozymes relative to the other PKC isozymes and other protein kinases are superior therapeutic agents. Such compounds should demonstrate greater efficacy and lower toxicity by virtue of their specificity.

The microbial indolocarbazole, staurosporine, is a potent inhibitor of protein kinase C that interacts with the catalytic domain of the enzyme. Tamaoki et al., Biochem. Biophys. Res. Commun. 135: 397-402 (1986); Gross et al., Biochem. Pharmacol. 40: 343-350 (1990). However, the therapeutic usefulness of this molecule and closely related compounds is limited by the lack of specificity for protein kinase C over other protein kinases. Ruegg, U.T. and

Burgess, G.M., Trends Pharmacol. Sci. 10: 218-220 (1989). This lack of selectivity results in unacceptable toxicity in this class of molecules.

An additional class of compounds related to staurosporine, the bisindolemaleimides, has been the focus of recent work. Davis et al., FEBS Lett. 259: 61-63 (1989); Twoemy et al., Biochem. Biophys. Res. Commun. 171: 1087-1092 (1990); Toullec et al., J. Biol. Chem. 266: 15771-15781 (1991); Davis et al., J. Med. Chem. 35: 994-1001 (1992); Bit et al., J. Med. Chem. 36: 21-29 (1993). Some of these compounds have demonstrated selectivity for protein kinase C over other protein kinases.

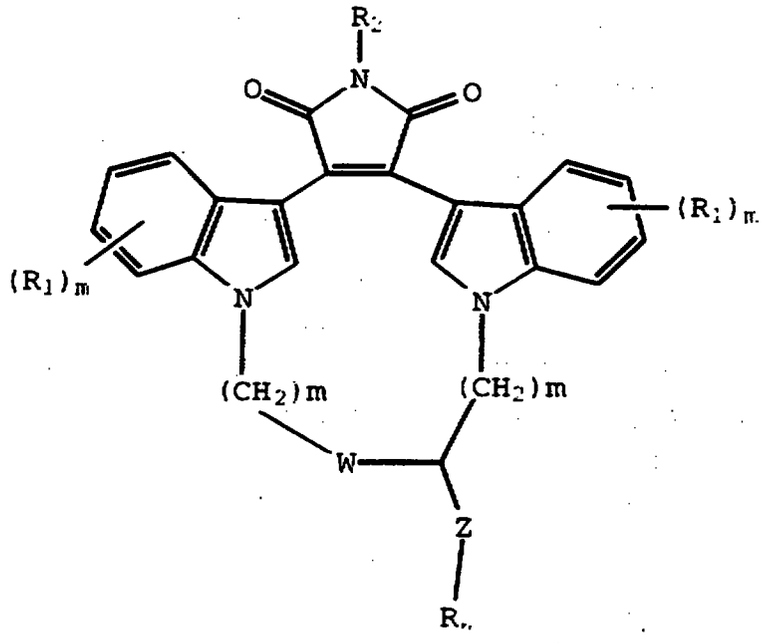
Although compounds that demonstrate specificity to protein kinase C have been discovered, very little is known regarding isozyme selectivity. For example, analysis of the isozyme selectivity of staurosporine, shows little isozyme selectivity with the exception of poor inhibition of the zeta isozyme relative to the other isozymes. McGlynn et al., J. Cell. Biochem. 49: 239-250 (1992); Ward, N.E., and O'Brian, C.A., Molec. Pharmacol. 41: 387-392 (1992). Studies of the PKC-selective compound, 3-[1-(3-dimethylaminopropyl)-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, suggest a slight selectivity for the calcium dependent isozymes. Toullec et al., J. Biol. Chem. 266: 15771-15781 (1991). Subsequent studies of this compound observed no difference, or possibly slight selectivity, for alpha over beta-1 and beta-2 isozymes. Martiny-Baron et al., J. Biol. Chem. 268: 9194-9197 (1993); Wilkinson, et al., Biochem. J. 294: 335-337 (1993). Therefore, despite years of research and the identification of classes of compounds that inhibit protein kinase C versus other protein kinases, there remains a need for therapeutically effective isozyme-selective inhibitors.

The present invention provides novel, potent protein kinase C inhibitors. The compounds of the present invention are selective to protein kinase C over other kinases and are, quite surprisingly, highly isozyme-selective. As selective inhibitors the compounds are useful

in treating conditions associated with diabetes mellitus and its complications, ischemia, inflammation, central nervous system disorders, cardiovascular disease, dermatological disease and cancer.

Summary of the Invention

This invention provides compounds of Formula I:



(I)

wherein:

W is O, -S-, or NH;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)$_2$, or -NHCO(C_1-C_4 alkyl);

R_2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

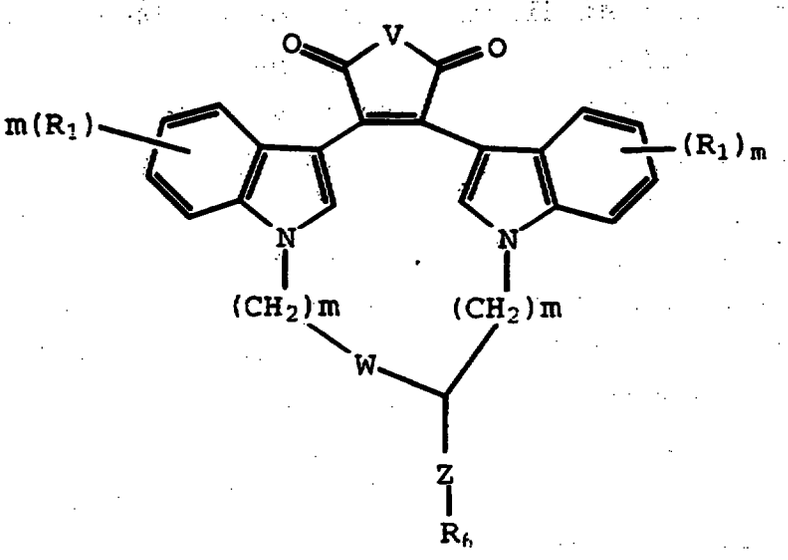
Z is -$(CH_2)_p$- or -$(CH_2)_p$-O-$(CH_2)_p$-;

R_6 is -NH(CF_3) or -N(CF_3)(CH_3);

m is independently 0, 1, 2, or 3; and

p is independently 0, 1 or 2.

Also provided are novel intermediates of the above compounds. These intermediates are compounds of the Formula II.



(II)

wherein:

V is -O- or N-CH_3;

W is O, -S-, or -NH;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)$_2$, or -NHCO(C_1-C_4 alkyl);

Z is -$(CH_2)_p$- or -$(CH_2)_p$-O-$(CH_2)_p$-;

R_6 is -NH(CF_3) or -N(CF_3)(CH_3);

m is independently 0, 1, 2, or 3; and

p is independently 0, 1 or 2.

One further aspect of the invention is a method of inhibiting Protein Kinase C, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I. Also included is a method of selectively inhibiting the beta-1 and beta-2 protein kinase C isozymes, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I.

The invention further provides methods for treating conditions that protein kinase C has demonstrated a role in the pathology, such as ischemia, inflammation, central nervous system disorders, cardiovascular disease,

dermatological disease, and cancer, which comprise administering to a mammal in need of treatment a pharmaceutically effective amount of a compound of the Formula I.

This invention is particularly useful in treating diabetic complications. Therefore, this invention further provides a method for treating diabetes mellitus, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I.

A final aspect of the invention are pharmaceutical formulations comprising a compound of Formula I together with one or more pharmaceutically acceptable excipients, carriers, or diluents.

Detailed Description and Preferred Embodiments

For purposes of the present invention, as disclosed and discussed herein, the following terms and abbreviations are defined as follows:

The term "halo" represents fluorine, chlorine, bromine, or iodine.

The term "C_1-C_4 alkyl" represents a cyclo, straight or branched chain alkyl group having from one to four carbon atoms such as methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl and the like. A haloalkyl is one such alkyl substituted with one or more halo atoms. An example of a haloalkyl is trifluoromethyl.

A C_1-C_4 alkoxy is a C_1-C_4 alkyl group covalently bonded to the parent moiety by an -O- linkage.

The term "leaving group" as used in the specification is understood by those skilled in the art. Generally, a leaving group is any group or atom that enhances the electrophilicity of the atom to which it is attached for displacement. Preferred leaving groups are triflate, mesylate, tosylate, imidate, chloride, bromide, and iodide. If the alkylating agent contains an amino acid residue (i.e.,

X, W, and Y combine to form -$(CH_2)_n$-AA-) the leaving group attached to the carboxy is preferably pentaflourophenyl ester or para-nitrophenyl ester.

The term "carboxy protecting group" as used in the specification refers to one of the ester derivatives of the carboxylic acid group commonly employed to block or protect the carboxylic acid group while reactions are carried out on other functional groups on the compound. The species of carboxy-protecting group employed is not critical so long as the derivatized carboxylic acid is stable to the condition of subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. T.W. Greene and P. Wuts, Protective Groups in Organic Synthesis, John Wiley and Sons, New York, N.Y., 1991, Chapter 5, provide a list of commonly employed protecting groups. See also E. Haslam, Protective Groups in Organic Chemistry, J.G.W. McOmie, Ed., Plenum Press, New York, N.Y., 1973. A related term is "protected carboxy," which refers to a carboxy-protecting group.

The term "hydroxy protecting group" as used in the specification refers to one of the ether or ester derivatives of the hydroxy group commonly employed to block or protect the hydroxy group while reactions are carried out on other functional groups on the compound. The species of hydroxy protecting group employed is not critical so long as the derivatized hydroxy group is stable to the condition of subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. T.W. Greene and P. Wuts, Protective Groups in Organic Synthesis, John Wiley and Sons, New York, N.Y., 1991, provide a list of commonly employed protecting groups. Preferred hydroxy protecting groups are tert-butyldiphenylsilyloxy (TBDPS), tert-butyldimethylsilyloxy (TBDMS), triphenylmethyl (trityl), methoxytrityl, or an alkyl or aryl ester. A related term is "protected hydroxy," which refers to a hydroxy protecting group.

The term "amino protecting group" as used in the specification refers to substituents of the amino group commonly employed to block or protect the amino functionality while reacting other functional groups on the compound. The species of amino-protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. T. W. Greene and P. Wuts, Protective Groups in Organic Synthesis, Chapter 7, provide a list of commonly employed protecting groups. See also J. W. Barton, Protective Groups in Organic Chemistry, Chapter 2. Preferred amino-protecting groups are t-butoxycarbonyl, pthalimide, a cyclic alkyl, and benzyloxycarbonyl. The related term "protected amino" defines an amino group substituted with an amino protecting group as defined.

The term "-NH protective groups" as used in the specification refers to sub-class of amino protecting groups that are commonly employed to block or protect the -NH functionality while reacting other functional groups on the compound. The species of protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. T. W. Greene and P. Wuts, Protective Groups in Organic Synthesis, Chapter 7, page 362-385, provide a list of commonly employed protecting groups. Preferred -NH protecting groups are carbamate, amide, alkyl or aryl sulfonamide. The related term "protected -NH" defines a group substituted with an -NH protecting group as defined.

The term "pharmaceutically effective amount", as used herein, represents an amount of a compound of the invention that is capable of inhibiting PKC activity in mammals. The particular dose of the compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the

particular condition being treated, and similar considerations. The compounds can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous, topical, intravenous, intramuscular or intranasal routes. For all indications, a typical daily dose will contain from about 0.01 mg/kg to about 20 mg/kg of the active compound of this invention. Preferred daily doses will be about 0.05 to about 10 mg/kg, ideally about 0.1 to about 5 mg/kg. However, for topical administration a typical dosage is about 1 to about 500 µg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu g/cm^2$, more preferably, from about 50 to about 200 $\mu g/cm^2$, and, most preferably, from about 60 to about 100 $\mu g/cm^2$.

The term "treating," as used herein, describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a compound of present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder.

The term "isozyme selective" means the preferential inhibition of protein kinase C beta-1 or beta-2 isozyme over protein kinase C isozymes, alpha, gamma, delta, epsilon, zeta, and eta. In general, the compounds demonstrate a minimum of a eight fold differential (preferably a ten fold differential) in the dosage required to inhibit PKC beta-1 or beta-2 isozyme and the dosage required for equal inhibition of the alpha protein kinase C isozyme as measured in the PKC assay. The compounds demonstrate this differential across the range of inhibition and are exemplified at the IC_{50}, i.e., a 50% inhibition. Thus, isozyme-selective compounds inhibit the beta-1 and beta-2 isozymes of protein kinase C at much lower concentrations with lower toxicity by virtue of their minimal inhibition of the other PKC isozymes.

As noted above, the invention provides compounds of the Formula I which selectively inhibit protein kinase C.

The preferred compounds of this invention are those compounds of Formula I wherein Z is $-(CH_2)_p-$; and R_6 is $NH(CF_3)$ or $N(CH_3)(CF_3)$, or m is independently 0, 1, 2 or 3, and p is 1.

By virtue of their acidic moieties, the compounds of Formula I include the pharmaceutically acceptable base addition salts thereof. Such salts include those derived from inorganic bases such as ammonium and alkali and alkaline earth metal hydroxides, carbonates, bicarbonates, and the like, as well as salts derived from basic organic amines such as aliphatic and aromatic amines, aliphatic diamines, hydroxy alkamines, and the like. Such bases useful in preparing the salts of this invention thus include ammonium hydroxide, potassium carbonate, sodium bicarbonate, calcium hydroxide, methylamine, diethylamine, ethylenediamine, cyclohexylamine, ethanolamine and the like.

Because of the basic moiety, the compounds of Formula I can also exist as pharmaceutically acceptable acid addition salts. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para- bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, mono-hydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-1,4 dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β-hydroxybutyrate, glycollate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like salts.

In addition to pharmaceutically-acceptable salts, other salts are included in the invention. They may serve as intermediates in the purification of the compounds, in the preparation of other salts, or in the identification and characterization of the compounds or intermediates.

The pharmaceutically acceptable salts of compounds of Formula I can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent. Such solvates are within the scope of the present invention.

It is recognized that various stereoisomeric forms of the compounds of Formula I may exist; for example, W may contain a chiral carbon atom in the substituted alkylene moiety. The compounds are normally prepared as racemates and can conveniently be used as such, but individual enantiomers can be isolated or synthesized by conventional techniques if so desired. Such racemates and individual enantiomers and mixtures thereof form part of the present invention.

The invention also encompasses the pharmaceutically acceptable prodrugs of the compounds of Formula I. A prodrug is a drug which has been chemically modified and may be biologically inactive at its site of action, but which may be degraded or modified by one or more enzymatic or other *in vivo* processes to the parent bioactive form. This prodrug should have a different pharmacokinetic profile than the parent, enabling easier absorption across the mucosal epithelium, better salt formation or solubility, and/or improved systemic stability (an increase in plasma half-life, for example). Typically, such chemical modifications include the following:

1) ester or amide derivatives which may be cleaved by esterases or lipases;

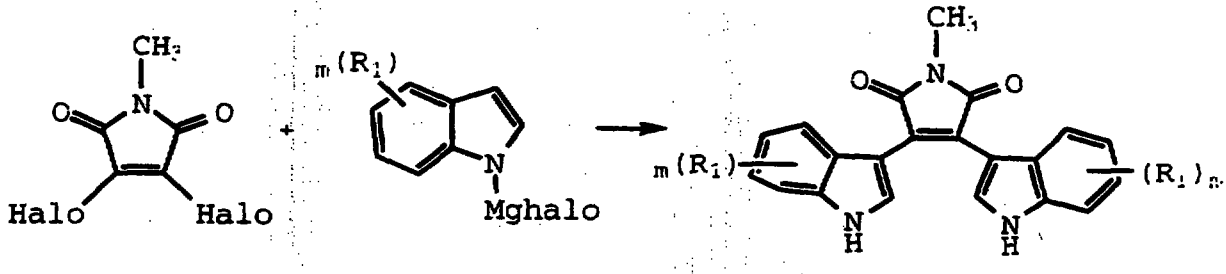
2) peptides which may be recognized by specific or nonspecific proteases; or

3) derivatives that accumulate at a site of action through membrane selection of a prodrug form or a modified prodrug form; or any combination of 1 to 3, supra.

Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in H, Bundgaard, Design of Prodrugs, (1985).

The synthesis of certain bis-indole-N-maleimide derivatives is described in Davis et al. U.S. Patent 5,057,614, herein incorporated by reference. Generally, the compounds of the present invention may be prepared as follows:

Scheme 1

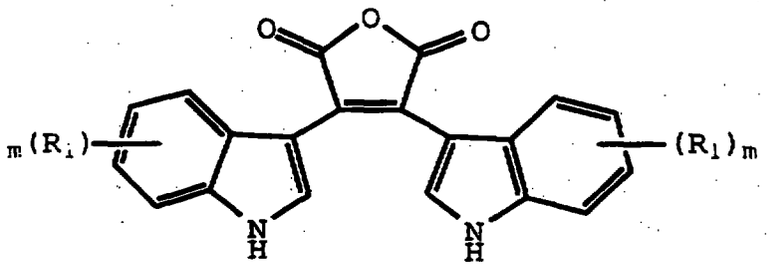


(III) (IV) (V)

R_1, m, and halo are the same as previously defined. Halo is preferably chloro, bromo, or iodo. Compound III is preferably 2,3-dichloro N-methylmaleimide.

The reaction between Compound III and the indole, Compound IV, is commonly known as a Grignard reaction. The reaction is carried out in an inert organic solvent, such as toluene, at a temperature between room temperature and the reflux temperature of the reaction mixture. Most significantly, the reaction depicted in Scheme 1 is dependent on solvent conditions. When carried out in a Toluene:THF:ether solvent system, the reaction provides Compound V in greater than 80 percent yield and greater than 95 percent purity. The product is precipitated from the reaction mixture with ammonium chloride, NH_4Cl. The resulting intermediate, Compound V, may be isolated by standard techniques.

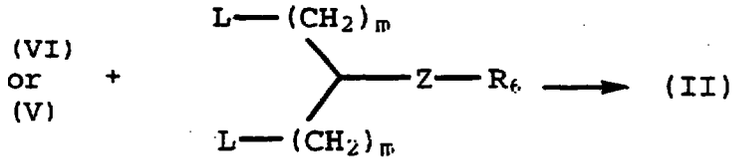
Bis-3,4(3'-indolyl)-1N-methyl-pyrrole-2,5-dione, Compound V, may then be converted by alkaline hydrolysis to the corresponding anhydride of the Formula VI by techniques known in the art and described in Brenner et al., Tetrahedron 44: 2887-2892 (1988). Preferably, Compound V is reacted with 5 N KOH in ethanol at a temperature ranging from 25°C to reflux.



(VI)

Compounds of the Formula V are generally more stable than the compounds of the Formula VI. Therefore, it is preferred that Compounds V are reacted in accordance with Scheme 2 to produce the compounds of Formula I. However, one skilled in the art would recognize that the compounds of the Formula VI, may also be reacted according to Scheme 2.

Scheme 2



(VII)

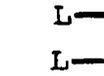
Z, R_6, and m are the same as previously defined. L is a good leaving group such as chloro, bromo, iodo, mesyl, tosyl, and the like. L may also be a hydroxy or other precursor that may be readily converted to a good leaving group by techniques known in the art. For example, the hydroxy may be readily converted to a sulfonic ester such as mesyl by

reacting the hydroxy with methanesulfonyl chloride to produce the mesylate leaving group.

The reaction represented by Scheme 2 is accomplished by any of the known methods of preparing N-substituted indoles. This reaction usually involves approximately equimolar amounts of the two reagents, although other ratios, especially those wherein the alkylating reagent is in excess, are operative. The reaction is best carried out in a polar aprotic solvent employing an alkali metal salt or other such alkylation conditions as are appreciated in the art. When the leaving group is bromo or chloro, a catalytic amount of iodide salt, such as potassium iodide may be added to speed the reaction. Reaction conditions include the following: Potassium hexamethyldisilazide in dimethylformamide or tetrahydrofuran, sodium hydride in dimethylformamide.

Preferably, the reaction is carried out under slow reverse addition with cesium carbonate in either acetonitrile, dimethylformamide (DMF), or tetrahydrofuran (THF). The temperature of the reaction is preferably from about ambient temperature to about the reflux temperature of the reaction mixture.

One skilled in the art would recognize that the reaction described in Scheme 2 may be employed with compounds of the Formula VIIa:



VIIa

X' and Y' are a protected carboxy, protected hydroxy, or a protected amine. After the alkylation of Scheme 2, X' and Y' may be converted to moieties capable of coupling to form the claimed compounds. This method is the preferred method of preparing the compounds of Formula I wherein W is -S-, -O-, or NH. The coupling of X' and Y' to form the various ether,

thioether or aminoether derivatives is known in the art and described in, for example, Ito, et al., Chem. Pharm. Bull. 41(6): 1066-1073 (1993); Kato, et al., J. Chem. Pharm. Bull. 34: 486 (1986); Goodrow, et al. Synthesis 1981: 457; Harpp, et al., J. Am. Chem. Soc. 93: 2437 (1971); and Evans, et al., J. Org. Chem. 50: 1830 (1985).

One skilled in the art would also recognize that the reaction of Scheme 2 may be carried out using appropriate protecting groups as a two step synthesis. That is, using appropriate protecting groups alkylation of each indolyl nitrogen of the compound of Formula V and VI may proceed as described herein in two steps (alkylating one indolyl under slow reverse addition and then closing the macrocycle by alkylation of the second indolyl.)

Most unexpectedly, the compounds of the invention may be prepared in substantially higher yield when the alkylation is carried out under slow reverse addition to Cs_2CO_3 in a polar aprotic solvent. Slow reverse addition involves combining a mixture of compound and alkylating agent with the base at a rate from about 0.1 mL/hour to about 2.0 mL/hour. The concentration of each reagent in the mixture is about 1.5 molar to about 0.001 molar. The slow addition results in a concentration of reagents in the reaction vessel of about 0.01 μmolar to 1.5 molar. One skilled in the art would recognize that at a higher rate of addition a lower concentration of reagents could be used in the reaction. Likewise, at a slower rate of addition, a higher concentration of reagents could be used in the reaction. Preferably, the compound is added at about .14 mL/hour with the compound and the alkylating agent at 0.37 molar. It is preferred that the Cs_2CO_3 be added in excess -- most preferably a 4:1 ratio Cs_2CO_3 to alkylating agent. Preferred polar aprotic solvents are acetonitrile, dimethylformamide (DMF), acetone, dimethylsulfoxide (DMSO), dioxane, diethylene glycol methyl ether (diglyme), tetrahydrofuran (THF), or other polar aprotic solvents in which the reagents are soluble. The reaction is carried out at temperatures ranging

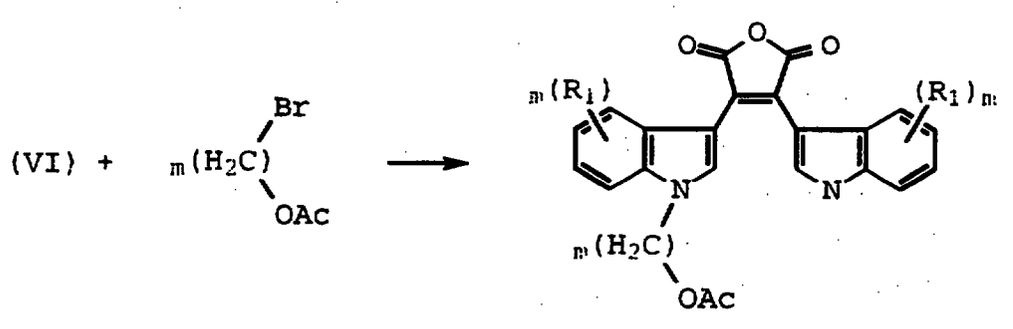
from about 0°C to reflux. One skilled in the art would recognize that the ratio of the mixture of the compound and alkylating agent is not critical. However, it is preferred that the reagents are mixed in a ratio of 0.5 to 3 equivalents of each other. Most preferably, the reagents are mixed 1:1.

When V is N-CH_3, Compound II is converted to the corresponding anhydride (V is O) by alkaline hydrolysis. Alkaline hydrolysis involves reacting the compound with a base, such as sodium hydroxide or potassium hydroxide, in C_1-C_4 alcohol (preferably ethanol), DMSO/water, dioxane/water, or acetonitrile/water at a temperature ranging from about 25°C to preferably about reflux. The concentration of the reactants is not critical.

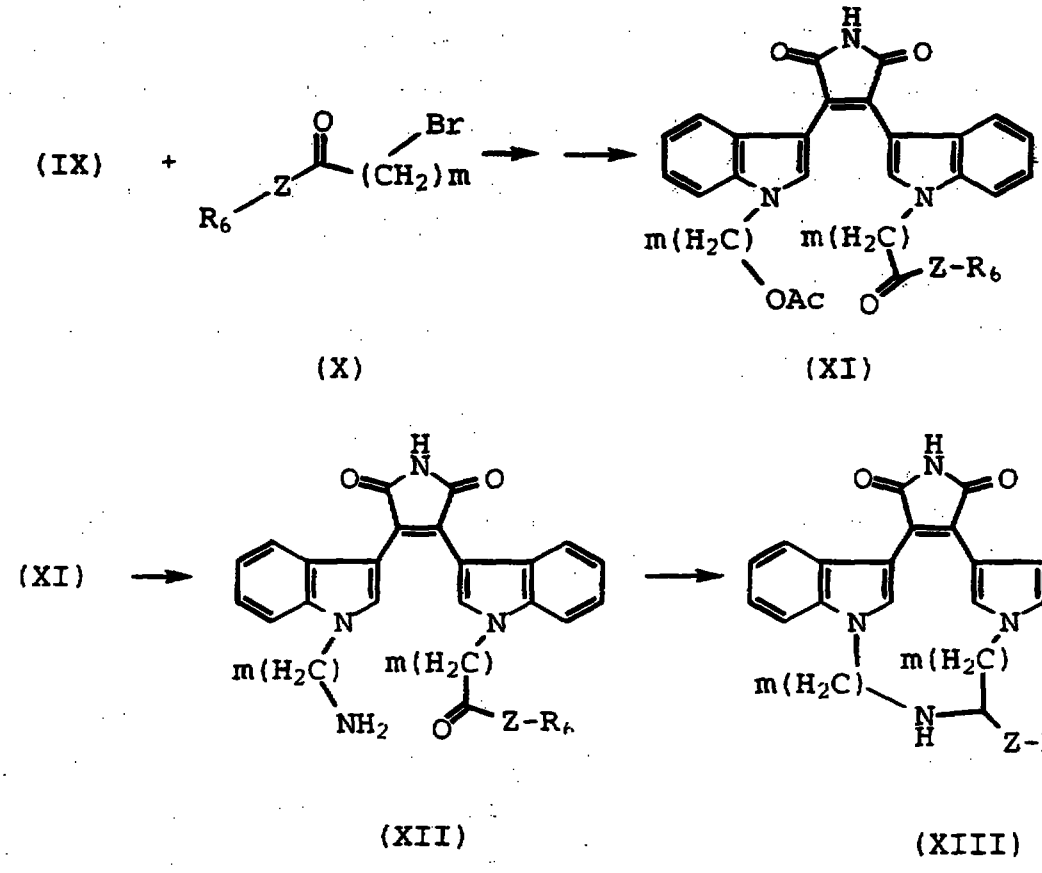
The anhydride (V is O) is converted to the maleimide of Formula I by ammonolysis. Ammonolysis involves reacting the anhydride with an excess of hexamethyldisilazane or an ammonium salt (ammonium acetate, bromide, or chloride) and C_1-C_4 alcohol (preferably methanol) in an polar aprotic solvent such as DMF at room temperature. Preferably, the hexamethyldisilazane or an ammonium salt is reacted at a ratio greater than about 5:1 equivalents of anhydride.

Yet another method of preparing the compounds of Formula I is outlined in Scheme 3. This method is particularly useful when W is -NH.

Scheme 3



(VIII) (IX)



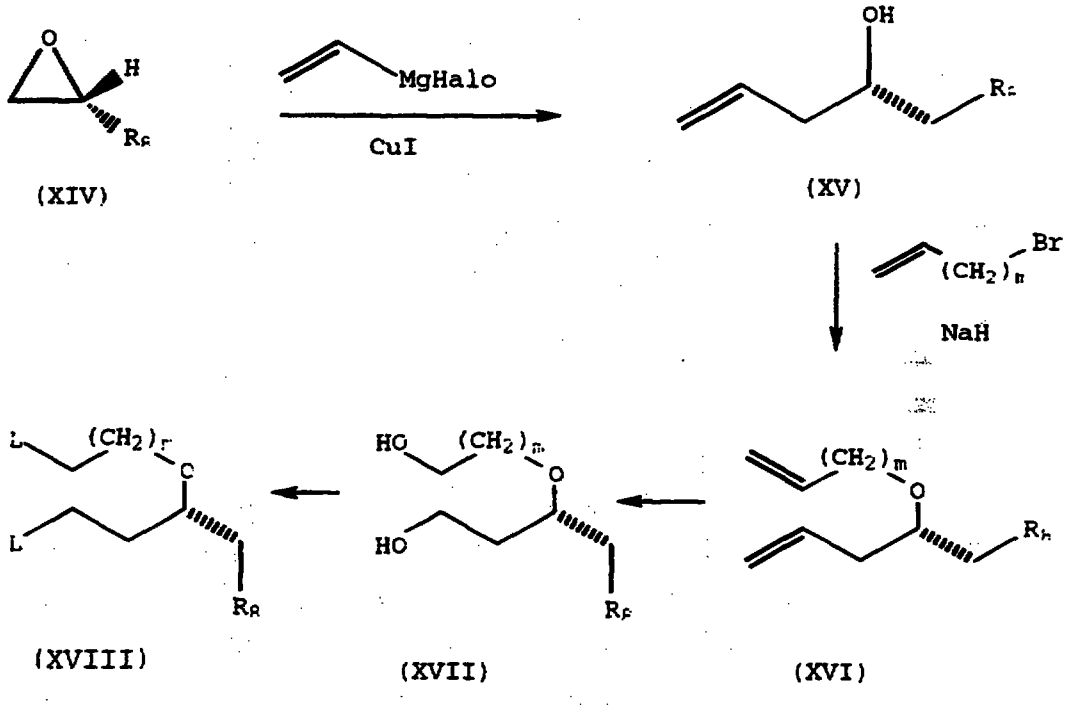
Ac is acetyl. R_6, Z, and m are the same as previously defined. The alkylation of Compound VI and VIII occurs under conditions previously described and know in the art. The alkylation of Compound IX with the α-halo ketone, Compound X, occurs under conditions previously discussed. The conversion of the anhydride to the maleimide, Compound XI, occurs as previously described. For example, the anhydride may be converted to the bis-indole maleimide by reacting the anhydride with hexamethyldisilazane and methanol in an inert organic solvent such as DMF at room temperature.

The protected hydroxy, represented by OAc, is readily hydrolyzed to form an alcohol (for example, K_2CO_3 in aqueous methanol and THF). The resulting alcohol is converted to a leaving group by methods appreciated in the art such as reacting the alcohol with mesyl chloride in triethylamine at 0°C. The leaving group is substituted with an azide, such as NaN_3 in DMF at 50°C. The resulting azide

is reduced to form the amine by employing Lindlar's catalyst in the presence of H_2. The macrocycle is allowed to close via an intramolecular Schiff base. The Schiff base is reduced under standard conditions, such as $NaCNBH_3$ or other reducing agents, to form the macrocycles of Formula I.

An intermediate of the present invention is prepared in accordance with Scheme 4. This scheme is particularly useful in preparing compounds wherein W is -O-.

Scheme 4



R_8 is N_3, NH-protecting group, amine protecting group, or hydroxy protecting group; m is independently 0, 1, 2, or 3; and L is a good leaving group such as chloro, bromo, iodo, mesyl, tosyl and the like. L is preferably mesyl. R_8 is preferably a protected hydroxy, most preferably -Otrityl. Scheme 4 presents a stereoselective synthesis of the linker portion of the macrocycle. The S-enantiomer is illustrated above; however, one skilled in the art would recognize that the complimentary enantiomer or mixture of enantiomers could be prepared in an analogous manner. Furthermore, one skilled in the art would recognize that an analogous reaction with a

methyl substituted epoxide or Grignard reagent could be used to prepare the various linkers containing a methyl substituted alkylene.

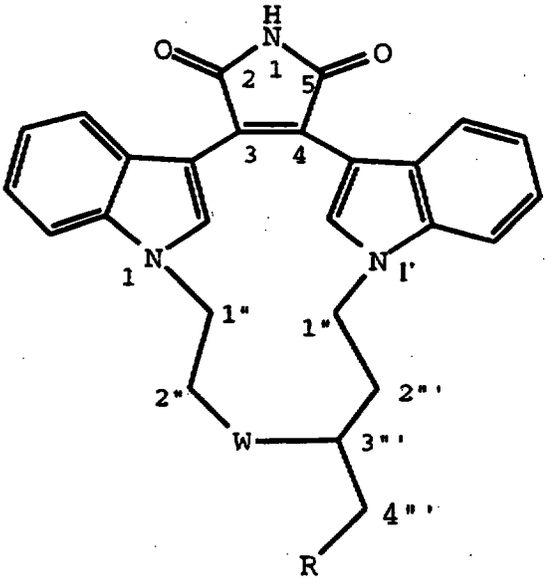
In the above reaction, the epoxide, Compound (XIV), is opened using a Grignard reagent. The reaction is carried out in the presence of copper complexing agent; however other alkylating conditions are operative. The reaction is carried out in an inert solvent at a temperature between -30°C and reflux temperature of the reaction mixture. The reaction produces Compound (XV) which may be further reacted without purification. Compound (XV) is allylated under general conditions known in the art for preparing ethers. The reaction illustrated in Scheme 4 is a Williamson synthesis. The formation of sodium alkoxide using NaH, NaOH, or KOH followed by allylation with allyl bromide produces the diene, Compound (XVI). Compound (XVI) is converted to the alcohol, Compound (XVII), under standard techniques. For example, Compound (XVI) can be converted to an ozonide by treating with ozone at low temperatures. The ozonide is then reduced with $NaBH_4$, $LiAlH_4$, BH_3 or catalytic hydrogenation with excess H_2 to produce the alcohol, Compound (XVII). The hydroxy moieties of Compound (XVII) are converted to leaving group, L, by standard techniques such as reacting the alcohol with mesyl chloride in triethylamine.

In all of the above schemes, it is preferred that the reactions be carried out with appropriate protecting groups. In particular, it is preferred that R_1 is protected during the alkylations and/or acylations and subsequently deprotected. Likewise, if R_6 is to be a $-NH(CF_3)$, the reactions are best carried out with an amino protecting group. However, one skilled in the art recognizes that many of these reactions can be performed without protecting groups if the appropriate reaction conditions, blocking reagents, or the like are used. It is preferred that when nacrocycle contains a hydroxy moiety, it is protected as *tert*-butyldiphenylsilyloxy (TBDPS) or triphenylmethyl (trityl) during the alkylation or acylation of the indole. The

resulting compounds of Formula I may be isolated and purified by standard techniques.

Compounds of Schemes 1-4 and any other reagents required for the above reactions, are either commercially available, known in the art, or can be prepared by methods known in the art. For example, Compound III may be prepared by techniques described in Edge et al., Chem. and Ind. 130 (1991); Compound IV is preferably prepared *in situ* by reacting an appropriately substituted indole with an alkylmagnesium halide such as ethylmagnesium bromide in a known manner.

The following examples and preparations are provided merely to further illustrate the invention. The scope of the invention is not construed as merely consisting of the following examples. To aid one skilled in the art, the following structure is provided to illustrate with a representative compound the nomenclature adopted herein:



In the following examples and preparations, melting point, nuclear magnetic resonance spectra, mass spectra, high pressure liquid chromatography over silica gel, N,N-dimethylformamide, palladium on charcoal, tetrahydrofuran, and ethyl acetate are abbreviated M.Pt., NMR, MS, HPLC, DMF, Pd/C, THF, and EtOAc respectively. The terms "NMR" and "MS"

indicate that the spectrum was consistent with the desired structure.

Preparation 1

2,3-bis-(3'-indolyl)-furan-1,4-dione

Sodium ethoxide (3.56 g, 50 mmol) was added to a solution containing 2,3-dichloromaleic anhydride (5.56 g, 33.3 mmol) and methylamine hydrochloride (3.50g, 55.0 mmol) in 40 mL of acetic acid. The mixture was stirred under a $CaCl_2$ drying tube at 25°C for 16 hours and then refluxed for 4 hours. The cooled mixture was poured into water (350 mL) and extracted with EtOAc (3 x 75 mL). The combined organic extracts were washed with 100 mL portions of saturated aqueous $NaHCO_3$, water and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure. The residue was recrystallized from ethanol to give 3.82 g (64%) of 2,3-dichloro N-methylmaleimide as white crystals. Concentration of the mother liquor and chromatography of the residue by radial preparative layer chromatography (Chromatotron, Harrison Research), gave an additional 0.81 g of 2,3-dichloro N-methylmaleimide, raising the yield to 77%.

A solution of indole (10.5 g, 90 mmol) in 175 mL of dry toluene was treated dropwise over 1 hour under N_2 with a solution of ethylmagnesium bromide (1.0M in THF, 90 mL, 90 mmol). After the addition was complete, the light-green solution was heated at 40°C for 30 minutes and then cooled to 25°C. A solution of 2,3-dichloro N-methylmaleimide (3.8 g, 21 mmol) in 50 mL of toluene was added over a 30-minute period. The reaction mixture was heated at 100°C for 3 hours, then cooled to 25°C, and quenched with 100 mL of 20 percent aqueous citric acid. The layers were separated. The aqueous phase was extracted with EtOAc (50 mL). The combined organic layers were dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was taken up in 30 mL of acetone and allowed to stand at 5°C for 40 hours. The solids were collected and washed with ice-cold

ether to give 5.25 g (73 percent) of 3,4-bis-(3'-indolyl)-1-methyl-pyrrole-2,5-dione as a red solid, M.Pt. 276-278°C.

To a solution of 3,4-bis-(3'-indolyl)-1-methyl-pyrrole-2,5-dione in 150 mL of ethanol was added 5N KOH (50 mL). The mixture was stirred 4 hours at 25°C and diluted with 150 mL of water. Most of the ethanol was evaporated under reduced pressure. The mixture was then acidified to pH 1. The precipitated product was filtered and washed with water. The crude product was dissolved in a minimum of CH_2Cl_2 and slowly filtered through a two-inch column of silica gel eluting with 50 percent EtOAc in hexane to give the titled compound (3.10 g 79 percent) as a red solid. M. Pt. 225-228°C.

Preparation 2

1-(*tert*-butyldimethylsilyloxy)-4-(*tert*-butyldiphenylsilyloxy)-butan-3-ol

To an anhydrous CH_2Cl_2 (110 mL) solution of 3-buten-1-ol (15 g, 0.21 mol) was added imidazole (28.6 g, 0.42 mol, 2 eq), followed by *tert*-butyldimethylsilyl chloride (32 g, 0.22 mol). After 90 minutes, the reaction was complete as indicated by TLC (10% EtOAc/hexane). The CH_2Cl_2 solution was transferred to a separatory funnel, diluted with CH_2Cl_2 (110 mL), washed with water (200 mL), and brine (200 mL). The organic layer was collected, dried over $MgSO_4$, filtered, and the solvent removed to yield an oil (1-(O-TBDMS)-3-butene) which was taken on to the next reaction. MS

The above oil was dissolved in a mixture of acetone (400 mL) and water (50 mL). *N*-Methylmorpholine-*N*-oxide (85.2 g, 0.63 mol, 3 eq) was then added. The resulting slurry was cooled to 0° C, and after 10 minutes a catalytic amount of OsO_4 (0.3 g) was added. The resulting slurry was allowed to stir overnight, gradually warming to room temperature. TLC (25% EtOAc/hexane) indicated the reaction was complete. The reaction mixture was quenched with sodium bisulfite, diluted with ether (1 L), washed with water (400 mL), and brine (400 mL). The organic layer was collected.

The aqueous layer extracted with ether (2 x 500 mL). The combined organic layers were dried, filtered, and concentrated to yield 4-(O-TBDMS)-1,2-butanediol as an oil, which was taken on to the next reaction.

The above oil was dissolved in anhydrous CH_2Cl_2 (250 mL). Imidazole (30 g, 0.44 mol, 2.5 eq) was added to the solution as a solid with stirring. The resulting solution was cooled to 0°C. After cooling 15 minutes, a CH_2Cl_2 (50 mL) solution of *tert*-butyldiphenylsilyl chloride (50 g, 0.18 mol, 1 eq) was added dropwise over 45 minutes. After the addition was complete, stirring was continued at 0°C for 2.5 hours. The solution was transferred to a separatory funnel, diluted with CH_2Cl_2 (250 mL), washed with water, brine, dried over $MgSO_4$, and filtered. The solvent removed under reduced pressure to give the crude product as an oil. The crude product was purified by eluting (10% EtOAc/hexane) it through a short column of silica gel. The eluting solvent was removed *in vacuo* to leave a viscous oil of the titled intermediate. (78.1 g, 93 % overall yield). MS

Preparation 3

1-(*tert*-butyldimethylsilyloxy)-3-(3-iodopropyloxy)-4-(*tert*-butyldiphenylsilyloxy)-butane

To a methylene chloride (20 mL)/cyclohexane (100 mL) solution of the alcohol of Preparation 4 was added allyl trichloroacetimidate (17.82 g, 88 mmols, 2.2 eq) under an N_2 balloon followed by trifluoromethanesulfonic acid (50 µL/g of starting material, 0.92 mL). After 20 hours, the solution was filtered, and the filtrate was washed with saturated aqueous $NaHCO_3$, water, and then brine. The organic layer was collected and dried over $MgSO_4$. The solvent was removed to give an oil, which was purified by flash chromatography on silica gel eluting with hexanes and increasing the polarity of the mobile phase to 5% ethyl acetate in hexanes over several liters to yield 19.27 g of the allylic ether, 1-(*tert*-butyldimethylsilyloxy)-3-(propeneoxy)-4-(*tert*-

butyldiphenylsilyloxy)-butane as a light brown oil (97% yield). MS.

To a THF (60 mL) solution of the above allyl ether (14.16 g, 28.38 mmols, 1 eq) was added 9-BBN (9-borabicyclo[3.3.1]nonane, 0.5 M solution in THF, 60 mL, 30 mmols, 1.1 eq) dropwise under nitrogen. After 3 hours, TLC (10% EtOAc in hexanes) of the reaction showed that the starting material had been consumed. To this solution was added 3M aqueous NaOH (10.41 mL, 31.22 mmols, 1.1 eq) followed by slow (1.5 hr) dropwise addition of 30% hydrogen peroxide (10.3 mL, 90.82 mmols, 3.2 eq). The reaction temperature during the peroxide quench was kept below 50°C(ice bath).

After 30 minutes, sodium chloride was added until the solution was saturated. The organic layer was removed; the aqueous layer was extracted with ether; the combined organic layers were dried and filtered; and the filtrate concentrated to give an oil. The crude oil was purified by flash chromatography on silica gel eluting with 10% EtOAc/hexanes and increasing the polarity to 20% EtOAc/hexanes after about 1.5 liters of solvent to yield 9.53 g of a light yellow oil (65% yield). MS.

To an anhydrous 0°C ether (150 mL) solution of the above alcohol was added triethylamine (2.93 g, 28.91 mmols, 1.5 eq.) followed by dropwise addition of mesyl chloride (3.31 g, 28.91 mmols, 1.5 eq.) with vigorous stirring. After 3 hours at 0°C, TLC (10% EtOAc in hexanes) indicated the starting material was consumed. The reaction was diluted with ether, washed with water, brine, dried over $MgSO_4$, and the solvent removed. The resulting oil was passed through a pad of silica eluting with 25% EtOAc/hexanes, and the eluant was concentrated. To an acetone (200 mL) solution of the resulting oil was added $NaHCO_3$ (0.17 g, 1.93 mmols, 0.1 eq.), and NaI (28.88 g, 192.7 mmols, 10 eq.). After stirring 30 minutes at room temperature under a nitrogen atmosphere, the reaction was heated to 50 °C with a water bath. After 2.5 hours, TLC (10% EtOAc in hexanes) indicated that the mesylate

was consumed. The reaction mixture was diluted with ether (500 mL), washed with cold saturated aqueous Na_2SO_3, water, brine, dried (MgSO4), and the solvent removed. The resulting oil was passed through a pad of silica eluting with 5% EtOAc in hexanes to give the purified title compound 10.3 g as a colorless oil (85% yield).

Preparation 4

(±)3,4-[(N,N'-1,1'-(3''-3-*tert*-butyldiphenylsilyloxymethylene)hexane)-bis-(3,3'-indolyl)]-1(methyl)-pyrrole-2,5-dione

A DMF (50 mL) solution of bis-(3,3'-indolyl)]-1-(methyl)-pyrrole-2,5-dione (3.41 g, 10.0 mmol) containing the dibromide 3-*tert*-butyldiphenylsilyloxymethylene-1,6-dibromohexane (5.64 g, 11 mmol, prepared in a manner analogous to the benzoyl derivative in Preparation 2) was added using a syringe pump over a 15 hour period to a DMF (350 mL) slurry of Cs_2CO_3 (11.2 g, 34.3 mmol) at 60 °C. After 4 hours from completion of the addition, the reaction was cooled to room temperature, poured into water (1.5 L), and extracted with CH_2Cl_2 (3 x 300 mL). The organic phase was washed with water, dried, filtered and concentrated. The concentrate was purified by flash chromatography eluting with 10% to 25% ethyl acetate/hexane to give the macrocycle 3,4-[(N,N'-1,1'-(3''-3-*tert*-butyldiphenylsilyloxymethylene)hexane)-bis-(3,3'-indolyl)]-1(methyl)-pyrrole-2,5-dione 2.95 g (43% yield) as a red oil. MS

Preparation 5

(S)-methyl 4-tert-butyldiphenylsilyloxy-3-(allyloxy)butyrate

To a cyclohexane (400 mL) solution of (S)-methyl 4-tert-butyldiphenylsilyloxy-3-(hydroxy)butyrate (20.0 g, 53.7 mmol) was added allyl trichloroacetimidate (21.74 g, 107.4 mmol), followed by trifluoromethanesulfonic acid (1 mL, 50mL/g alcohol) in five portions over 30 minutes, with stirring under a nitrogen atmosphere. After 70 hours, the

solids that formed were filtered, and the filter cake was washed with cyclohexane, and the volatiles were removed *in vacuo*. The resultant oil was placed on a plug of silica and washed with hexane, and product eluted with 10% ethyl acetate/hexane. NMR indicated the presence of residual imidate (ca. 10%); however the material was carried on without further purification. The residue yields 24.76 g of material, of which approx. 22.2 g was desired product (100%). MS.

Preparation 6

(S)-4-tert-butyldiphenylsilyloxy-3-(2-iodoethoxy)-1-iodobutane

DIBAL-H (231 mL, 1.0M in toluene, 231 mmol) was added dropwise over 40 minutes to a solution of (S)-methyl 4-tert-butyldiphenylsilyloxy-3-(allyloxy)-butyrate (23.8g, 57 mmol) dissolved in anhydrous THF (1.0 L) at -75°C under N_2. After stirring 1.5 hours, the mixture was allowed to warm to -10°C and quenched with 5% water in methanol and a large amount of Celite. The quenched reaction mixture was filtered through a pad of Celite; the filtrate was concentrated and partitioned between ether and 20% citric acid. The ether layer was dried and concentrated *in vacuo*. The residual oil was passed through a pad of silica eluting with chloroform to yield 20.6 g (93%) of (S) 4-tert-butyldiphenylsilyloxy-3-allyloxy-butan-1-ol.

To a methanol (500 mL) solution of (S) 4-tert-butyldiphenylsilyloxy-3-allyloxybutan-1-ol (20.6 g, 53.6mmol) was added ozone at -78°C for approximately 12 minutes. The reaction mixture developed a faint blue color, $NaBH_4$ (12.2 g, 321 mmol, 6 eq.) was added to the reaction vessel. The reaction was allowed to come to room temperature. The volatiles were removed *in vacuo*. The residue was passed through a plug of silica eluting with ethyl acetate to yield 16.4 g (79%) of (S) 4-tert-butyldiphenylsilyloxy-3-(2-hydroxy-ethoxy)-butan-1-ol as a colorless oil.

To an ether (600 mL) solution of (S) 4-tert-butyldiphenylsilyloxy-3-(2-hydroxy-ethoxy)-butan-1-ol (15.7

g, 40.4 mmol) at 0°C under nitrogen was added triethylamine (16.8 mL, 121 mmol) followed by mesyl chloride (9.38 mL, 121 mmol). After 3 hours, the solution was filtered; the filtrate was washed with water (2x), brine (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The residue gave 21.9 g (>99%) of the bismesylate as a yellow oil which was carried on directly. The bismesylate was dissolved in acetone (1.4 l), which had been distilled from potassium carbonate. To this solution was added NaI (90.4 g, 603 mmol) and 0.05 eq. $NaHCO_3$ (170 mg, 2mmol). The reaction mixture was kept at 56°C for 24 hours and filtered; and the filtrate was concentrated *in vacuo*. The residue was partitioned between ether and 10% Na_2SO_3, the ether layer was washed with brine, dried over Na_2SO_4, and concentrated to give 17.9 g (73.2%) of (S)-4-tert-butyldiphenylsilyloxy-3-(2-iodoethoxy)-1-iodobutane as a colorless oil. The overall yield was 54%. MS: MW= 608.39; observed: 559 (M-*tert*butyl; FD, $CHCl_3$).

Preparation 7

(S)-3-(tert-butyldiphenylsilyloxymethylene)-1,6-dibromohexane

Following the same procedure described for the preparation of racemic dibromide, 3-(tert-butyldiphenylsilyloxymethyl)-1,6-dibromohexane, (S)-(-)-3-(tertbutyldiphenylsilyloxymethyl)-1,6-hexanediol (4.85 g, 12.53 mmol) was reacted with *N*-bromosuccinimide (5.35 g, 30.1 mmol) and triphenylphosphine (7.87 g, 30.1 mmol) CH_2Cl_2 (150 mL) at 0°C to afford compound (S)-(-)-3-(tert-butyldiphenylsilyloxymethyl)-1,6-dibromohexane 4.81 (75%) as a clear, colorless oil which was homogenous by TLC (R_f = 0.8, 10% EtOAc in hexanes. Both the TLC properties and ^1H spectra of this compound were identical in all respects with racemic isomer. MS.

^1H NMR (300 MHz, $CDCl_3$) 1.06 (s,9H), 1.35 - 2.10 (m, 7H), 3.55 (m,4H), 3.56 (app d, 2H, J = 4Hz), 7.40 and 7.64 (m, 10H).

Preparation 8

1-(tert-butyldimethylsilyloxy)-3-(2-iodoethoxy)-4-(tert-butyldiphenyl)-butane

The allyl ether, 1-(tert-butyldimethylsilyloxy)-3-(allyloxy)-4-(tert-butyldiphenyl)-butane, (21.6 g, 43.4 mmol) was dissolved in methanol (500 mL) and cooled to -78 °C under nitrogen. Ozone was bubbled into the reaction and after 11 minutes it was judged complete by TLC(9 hexane/1 ethyl acetate). Sodium borohydride (9.9 g, 6 eq) was added and after 5 minutes the reaction was allowed to warm to room temperature. The methanol was removed *in vacuo*. The residue was suspended in ether (800 mL). The ether was washed with water, and the aqueous backwashed with ether. The combined organics were washed with brine, dried ($Na2SO_4$), filtered and concentrated *in vacuo* to give an oil. The material was passed through a silica pad with 5% ethyl acetate/hexane followed by elution of the product with 25% ethyl acetate/hexane to provide 11.0 g (50% yield) of the alcohol, 1-(tert-butyldimethylsilyloxy)-3-(2-(hydroxy)ethoxy)-4-(tert-butyldiphenyl)-butane as a light yellow oil. MS. NMR.

To an anhydrous ether (200 mL) solution of the alcohol, 1-(tert-butyldimethylsilyloxy)-3-(2-(hydroxy)ethoxy)-4-(tert-butyldiphenyl)-butane, (11.0 g, 21.9 mmol) under nitrogen at 5 °C was added triethylamine (4.6 mL, 1.5 eq) and methanesulfonyl chloride (2.5 mL, 1.5 eq). After 1.5 hours the reaction was complete by TLC (5% ethyl acetate/dichloromethane). The reaction was diluted with ether (250 mL), washed with water (2X), brine (2X), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give an oil. The material was passed through a silica pad eluting with 5% ethyl acetate/hexane followed by 25% ethyl acetate/hexane to provide 11.6 g (91% yield) of the mesylate, 1-(tert-butyldimethylsilyloxy)-3-(2-(methanesulfonyloxy)ethoxy)-4-(tert-butyldiphenyl)-butane as an oil. MS. NMR.

To an acetone (300 mL) solution of the mesylate, 1-(tert-butyldimethylsilyloxy)-3-(2-(methanesulfonyloxy)ethoxy)-4-(tert-butyldiphenyl)-butane,

(11.6 g, 20 mmol) under nitrogen was added sodium iodide (44 g, 15 eq) and sodium bicarbonate (170 mg, 0.1 eq). The mixture was refluxed for 18 hours followed by removal of the acetone *in vacuo*. The resulting residue was suspended in ether, washed with water (2X), and the aqueous backwashed with ether. The combined ether portions were washed with 10% sodium sulfite solution, brine (2X), dried ($MgSO_4$), filtered and concentrated *in vacuo* to provide 10.7 g (87% yield) of the title iodide as an oil which was used without further purification. MS. NMR.

Preparation 9

3,4-[(N,N'-1,1'-((2''-ethoxy)-3''(O)-4''-(hydroxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione

To a dimethylformamide (250 mL) solution of bis-(3,3'-indolyl)-1-(methyl)-pyrrole-2,5-dione (17.9 g, 52.5 mmol, 3 eq) under nitrogen was added cesium carbonate (68.4 g, 4 eq). To the resulting suspension was added the iodide, 1-(*tert*-butyldimethylsilyloxy)-3-(2-iodoethoxy)-4-(*tert*-butyldiphenylsilyloxy)-butane, (10.7 g, 17.5 mmol). The reaction stirred for 18 hours at room temperature. TLC (5% ethyl acetate/hexane) showed disappearance of the iodide. The reaction was poured into ethyl acetate (1200 mL) and washed with 1N HCl (400 mL) followed by backwash with ethyl acetate (2X). The combined ethyl acetate portions were washed with saturated sodium bicarbonate solution, brine (2X), dried ($MgSO_4$), filtered and concentrated down *in vacuo*. Dimethylformate was removed by azeotroping with xylene. The resulting red gum was slurried in dichloromethane and acetonitrile to give a solid suspension. It was concentrated down, more dichloromethane added, cooled and filtered to give a red solid. Some of the desired product was extracted from this solid by another trituration in dichloromethane and then in ethyl acetate. The filtrates were concentrated *in vacuo* and the resulting residue absorbed on silica and applied to a large flash column. Dialkylated by-product was removed by elution with 5 hexane/1 ethyl acetate followed by elution of

the product with 3 hexane/1 ethyl acetate to provide 8.2 g (57%) of the monoalkylated product, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(tert-butyldimethylsilyloxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione. MS. NMR.

To a methanol (450 mL) solution of the tert-butyldimethylsilyl ether, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(tert-butyldimethylsilyloxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione (8.2 g, 9.9 mmol) under nitrogen at 5 °C was added *p*-toluenesulfonic acid, monohydrate (0.16 g, .085 eq). After 2 hours, TLC (50% ethyl acetate/hexane) showed the reaction to be nearly complete. The reaction was quenched with solid sodium bicarbonate (0.14 g). The methanol was removed *in vacuo*. The resulting residue was dissolved in ethyl acetate, washed with 0.1N sodium hydroxide, brine (2X), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a red foam. This material was absorbed on silica and placed on a silica pad. Elution with 2 hexane/1 ethyl acetate removed residual starting material followed by elution with 1 hexane/1 ethyl acetate and 1 hexane/2 ethyl acetate to provide 6.4 g(91%) of the alcohol, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(hydroxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione. MS. NMR.

To an anhydrous ether (500 mL) solution of the alcohol, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(hydroxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione (6.36 g, 8.9 mmol) under nitrogen at 5 °C was added triethylamine (1.9 mL, 1.5 eq) and methanesulfonyl chloride (1.0 mL, 1.5 eq). After 3 hours, additional triethylamine (1.25 mL, 1.0 eq) and methanesulfonyl chloride (0.7 mL, 1.0 eq) were added. After 1 hour, the reaction was shown to be complete by TLC (50% ethyl acetate/hexane). The reaction was diluted with ether (250 mL), washed with water, 0.1N HCl and brine (2X). The ether was dried ($MgSO_4$), filtered, and concentrated *in vacuo*

to provide 7.0 g of mesylate, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(methanesulfonyloxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione. MS.

To an acetone (200 mL) solution of the mesylate, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(methanesulfonyloxy)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione, (7.0 g, 8.9 mmol) under nitrogen was added sodium iodide (13.3 g, 10 eq) and sodium bicarbonate (75 mg, 0.1 eq). The mixture was stirred at 50°C for 13 hours. The reaction was concentrated in *vacuo*, and the residue was dissolved in ether and washed with 10% sodium sulfite solution. The layers were separated, and the ether portion washed with 10% sodium sulfite solution, water, brine(2X), dried, and concentrated *in vacuo*. The residue was passed through a silica pad by eluting with 1 hexane/1 ethyl acetate and 1 hexane/2 ethyl acetate to provide 7.6 g of the iodide, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(iodo)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione as a red solid (quantitative yield for the two steps). MS. NMR.

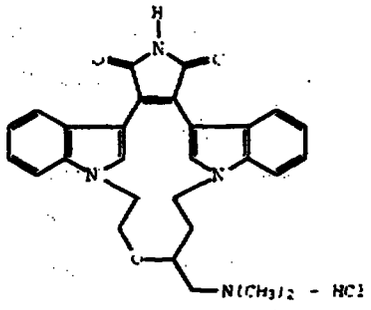
To a dimethylformamide (1 L) suspension of cesium carbonate (12.0 g, 4 eq) under nitrogen was added the iodide, 3-[(N-1-(2-ethoxy-(3'''-(O)-4'''-(tert-butyldiphenylsilyloxy)-1'''-(iodo)-butane))-indol-3-yl]-4-[indol-3-yl]-1N(methyl)-pyrrole-2,5-dione (7.6 g, 9.2 mmol), dissolved in dimethylformamide(25 mL) via syringe pump over 65 hours. Three hours after the addition was complete, the reaction was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (700 mL), washed with water (2 X 300 mL), and the aqueous layer backwashed with ethyl acetate (2 X 200 mL). The combined ethyl acetate portions were washed with brine (2 X 200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to provide a purple residue. The material was absorbed onto silica and applied to a flash column. Eluted with 3 hexane/1 ethyl acetate and then 1hexane/1 ethyl acetate to give 5.2 g(82%) of the macrocycle,

3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(tert-butyldiphenylsilyloxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione. MS. NMR.

A suspension of the N-methyl maleimide, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(tert-butyldiphenylsilyloxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione in 5N KOH (150 mL) and ethanol (300 mL) was stirred at room temperature for 65 hours and then for one hour at 60°C. The reaction was concentrated (150 mL) *in vacuo*, the residue suspended in water, cooled to 5 °C, and acidified (pH 3) with concentrated hydrochloric acid. The red aqueous suspension was extracted with ethyl acetate (4 X 200 mL), dried, and concentrated *in vacuo* to give 3.3 g of the crude anhydride alcohol, 2,3-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(hydroxy)-butane)-bis-(3,3'-indolyl)]-furan-1,4-dione as a purple solid. MS.

To a dimethylformamide (250 mL) solution of the anhydride, 2,3-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(hydroxy)-butane)-bis-(3,3'-indolyl)]-furan-1,4-dione, (3.3 g, 7.5 mmol) under nitrogen was added 1,1,1, 3, 3, 3 - hexamethyldisilazane (32 mL, 2 eq) and methanol (3 mL, 10 eq). The reaction was stirred at room temperature for 16 hours and then heated at 60°C for 2 hours. The dimethylformamide was removed *in vacuo*, and the resulting residue was dissolved in acetonitrile (250 mL). 1N HCl (50 mL) was added. The reaction was stirred for 15 minutes. The reaction was concentrated, partitioned between ethyl acetate (1 L) and water (250 mL). The product was a solid that precipitated giving the alcohol maleimide, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(hydroxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione, 0.92(28%) of product. A small amount (50 mg) was absorbed on silica and applied to a flash column. Eluted with dichloromethane, 5% acetonitrile/dichloromethane and then 10% acetonitrile/dichloromethane to give 38 mg of analytically pure material. The ethyl acetate was concentrated and chromatographed to give an additional 8% of the crude product. MS.

^{1}H NMR (d_6-DMSO): δ1.96 (1H, m); 2.09 (1H, m); 3.31 (1H, m); 3.40 (1H, m); 3.51 (1H, m); 3.62 (1H, m); 3.89 (1H, m); 4.18 (3H, m); 4.35 (1H, m), 4.68 (1H, t, J = 2 Hz); 7.11 (2H, m); 7.19 (2H, m); 7.44 (1H, s) 7.46 (1H, d, J = 9 Hz); 7.51 (1H, s) 7.53 (1H, d, J = 9 Hz); 7.79 (1H, d, J = 8 Hz); 7.83 (1H, d, J = 8 Hz); 10.91 (1H, s).



Preparation 10

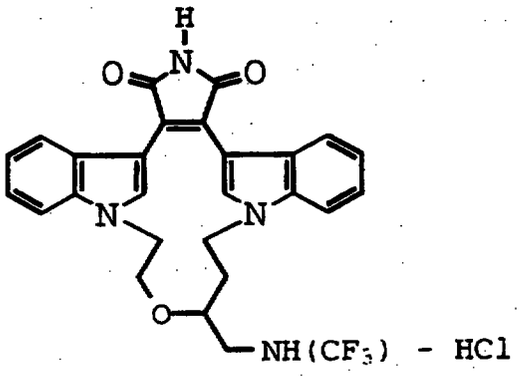
3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione HCl Salt

To an anhydrous dichloromethane (140 mL) suspension of the alcohol, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(hydroxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione, (472 mg, 1.07 mmol) under nitrogen was added pyridine (260 µL, 3 eq) and methanesulfonic anhydride (242 mg, 1.3 eq). After 4 hours, the reaction was diluted with dichloromethane, washed with 0.1N HCl (2X) and filtered to remove starting material (54 mg). The dichloromethane portion was washed with brine (2X), dried, and concentrated to give the crude mesylate, as a purple solid. The material was absorbed on silica and applied to a flash column which was sequentially eluted with dichloromethane, 5% acetonitrile/dichloromethane and 10% acetonitrile/ dichloromethane to provide 288 mg (52% yield) of the mesylate, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(methanesulfonyloxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione. MS. NMR.

To a tetrahydrofuran (20 mL) solution of the mesylate, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(methanesulfonyloxy)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione, (304 mg, 0.59 mmol) was added a 8.9 M solution of dimethylamine in tetrahydrofuran (7 mL, 100 eq). After heating (65 °C) for 24 hours in a sealed tube, the reaction was diluted with ethyl acetate (200 mL), washed with brine (2X), dried, and concentrated to provide the crude dimethylamine derivative as a solid. The material was absorbed on silica and applied to a flash column that was sequentially eluted with 3 ethyl acetate/ 1 hexane, ethyl acetate and 2% isopropylamine/ethyl acetate to give the dimethylamine derivative 193 mg (70% yield) which was 90% pure by HPLC. The dimethylamine derivative, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione,was purified to greater than 95% as the triflouroacetate salt using reverse phase size exclusion HPLC by eluting with 85 acetonitrile/15 (0.01%TFA/water).

The triflouroacetate salt of 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione was converted to the HCl salt by suspending the salt in ethyl acetate and washing gently with 0.1N NaOH(5 X 50 mL). The ethyl acetate portion was washed with brine (2X), dried, and concentrated to provide the free base, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione. To an anhydrous methanol (50 mL) suspension of the free base, 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione was added 1N HCl in anhydrous ether (13 mL, 50 eq). The ether was evaporated, and the residue was dried under vacuum to give 143 mg (52% yield) of 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione hydrochloride salt as a red solid. MS.

^1H NMR (d_6-DMSO): δ2.03 (1H, m); 2.26 (1H, m); 2.68 (6H, t, J = 5 Hz); 3.24 (1H, m); 3.28, (1H, m, after D_2O shake); 3.64 (1H, m); 3.77 (2H, m); 4.07 - 4.38 (4H, m); 7.08 (2H, m); 7.17 (2H, m); 7.43 (3H, m); 7.52 (1H, d, J = 8 Hz); 7.79 (2H, m); 10.33 (1H, bs); 10.92 (1H, s)



Example 1

3,4-[(N,N'-1,1'-((2''-ethoxy)-(3'''(O)-4'''-(N-trifluoromethylamino)-butane)-bis(3,3'-indolyl)]-1(H)-pyrrole-2,5-dione

3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N-methylamine)-butane-bis(3,3'-indolyl)]-1-(methyl)-pyrrole-2,5-dione prepared in a manner analogous to Preparation 10 (20 mg, 0.04 mmol) was dissolved in THF (10 mL) containing triethylamine (6.1 µL, 0.044 mmol) under nitrogen. To this solution was added carbon disulfide (3 microL, 0.05 mmol), and after 15 minutes methyl iodide was added. The reaction was complete after 12 hours by TLC (10% MeOH in CH_2Cl_2). The reaction mixture was diluted with ethyl acetate, washed with water, brine, dried and concentrated to give a dithiocarbamate (23 mg, expected mass) IS/MS 559 (M^++1) expected mass 558.

To a dichloromethane solution of the dithiocarbamate is added tetrabutylammonium dihydrogen-trifluoride and N-bromosuccinimide. Work up and purification by chromatography generates the 3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''-(N,N+(trifluoromethy)methylamino)-

butane-bis(3,3' indolyl)]-1-(methyl)-pyrrole-2,5,-dione, which is converted to the N-H maleimide.

The trifluoromethylamine derivative may also be prepared as follows:

To a DMSO solution of the monomethyl amine is added dibromodifluoromethane and tetrakis(dimethylamine)-ethylene. Standard work up gives the desired trifluoromethylamine derivative. The trifluoromethyl derivative would be converted to the N-H maleimide as previously described.

As previously noted, the compounds of the present invention are potent, protein kinase C inhibitors. The compounds are selective for protein kinase C over other kinases. The ability of the compounds of the present invention to selectively inhibit protein kinase C was determined in the Calcium Calmodulin Dependent Protein Kinase Assay, Casein Protein Kinase II assay, cAMP-Dependent Protein Kinase Catalytic Subunit assay and the Protein-Tyrosine Kinase assay.

Calcium Calmodulin Dependent Protein Kinase Assay (CaM)

The Calcium Calmodulin Dependent Protein Kinase Assay is described in the Journal of Neuroscience, 3:818-831 (1983). The assay components are in a total volume of 250 µL: 55 mM HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid), pH 7.5, 2.75 mM dithiothreitol, 2.2 mM EGTA (ethylenebis(oxyethylenenitrilo)tetraacetic acid, used in the blank buffer), 1.1 mM calcium chloride (Sigma, St. Louis, Missouri) (used in the control buffer), 10 mM magnesium chloride (Sigma, St. Louis, Missouri), 200 µg/mL histone type HL (Worthington), 10 µL DMSO or DMSO/inhibitor and 30 µM (gamma 32P) ATP (DuPont). The reaction is initiated by the addition of calcium calmodulin dependent protein kinase (isolated from rat brain homogenate), incubated at room temperature for 10 minutes and stopped by adding 0.5 mL ice cold trichloroacetic acid (Amresco) followed by 100 µL of 1 mg/mL bovine serum albumin (Sigma,

St. Louis, Missouri). The precipitate is collected by vacuum filtration on glass fiber filters and quantified by counting in a beta scintillation counter.

Buffer components:

	Control buffer	Blank buffer
200 mM HEPES pH 7.5	3125 µL	625 µL
50 mM DTT	625 µL	125 µL
histone	1250 µL	250 µL
100 mM calcium	125 µL	-----
100 mM EGTA	------	50 µL
DI water	2375 µL	450 µL

Assay components:

165 µL Buffer
25 µL calmodulin (250 µg/mL)
10 µL DMSO or DMSO/inhibitor
25 µL kinase enzyme
25 µL AT32P.

Casein Protein Kinase II Assay (CK-II)

The Casein Protein Kinase II Assay is described in Neurochem. Res., 13: 829-836 (1988). The assay components are in a total volume of 250 µL: 20 mM Tris-HCl, pH 7.5, 5 mM sodium fluoride, 50 mg/mL Casein (Sigma, St. Louis, Missouri), 10 mM magnesium chloride (Sigma, St. Louis, Missouri), 10 µL DMSO or DMSO/inhibitor and 30 µm (gamma-32P) ATP (DuPont). Initiation of the reaction is performed by addition of casein protein kinase II (isolated from rat brain homogenate), incubated at room temperature for 10 minutes and stopped by the addition of 0.5 mL ice cold Trichloroacetic acid (Amresco) followed by 100 µL of 1 mg/mL bovine serum albumin (Sigma, St. Louis, Missouri). The precipitate is collected by vacuum filtration on glass fiber filters and quantified by counting in a beta scintillation counter.

Assay components in order of addition

175 µL Buffer

10 µL or DMSO or DMSO/inhibitor

25 µL of AT32P in 300 µM magnesium chloride

40 µL of enzyme (undiluted)

Buffer prepared as follows:

(Final volume = 3.5 mL: amount of 20 assays)

500 µL of each:	200 mM Tris-HCl pH 7.5
	50 mM sodium fluoride
	50 mg/mL Casein
	+ 2 mL DI water
Total Volume	3.5 mL

cAMP-Dependent Protein Kinase Catalytic Subunit Assay (PKA)

The Assay components are in a total volume of 250 µL: 20 mM HEPES (Sigma, St. Louis, Missouri) buffer pH 7.5, 200 µg/mL histone type HL (Worthington), 10 mM magnesium chloride (Sigma, St. Louis, Missouri), 10 µL DMSO or DMSO inhibitor and 30 µM (gamma- 32P) ATP (DuPont). The reaction is initiated by addition of bovine heart cAMP-dependent kinase catalytic subunit (Sigma, St. Louis, Missouri), incubated to 30°C for 10 minutes and stopped by adding 0.5 mL ice cold Trichloroacetic acid (Amresco) followed by 100 µL of 1 mg/mL bovine serum albumin (Sigma). The precipitate is collected by vacuum filtrated on glass fiber filters employing a TOMTEC™ and quantified by counting in a beta scintillation counter. This assay is done identical to the protein kinase C (PKC) enzyme assay except that no phospholipids or diacylglycerol are employed in the assay and the histone substrate is specific for the cAMP-dependent catalytic subunit enzyme.

Protein Tyrosine Kinase Assay (src)

The Assay components are the following:

10 µL Raytide

10 µL Kinase
4 µL DMSO or DMSO/inhibitor
6 µL 200 mM HEPES pH 7.5
10 µL AT32P

This assay is described by Onogene Science, Inc. Cat. #PK02 and PK03 (1990).

Surprisingly, the compounds of the present invention are also isozyme-selective inhibitors, that is, the compounds selectively inhibit protein kinase C beta-1 and beta-2 isozymes. This isozyme selectivity was determined in the PKC Enzyme Assay.

PKC Enzyme Assay

PKC enzymes = alpha, beta I, beta II, gamma, delta, epsilon, eta and zeta.

Assay components in a total volume of 250 µL are as follows:

Vesicles consisting of 120 µg/mL phosphatidylserine (Avanti Polar Lipids) and sufficient diacylglycerol (Avanti Polar Lipids) to activate the enzyme to maximum activity in 20 mM HEPES buffer (Sigma, St. Louis, Missouri), pH 7.5, 940 µM calcium chloride (Sigma, St. Louis, Missouri) for assaying the alpha, beta-1, beta-2 and gamma enzyme only, 1 mM EGTA for all the enzymes, 10 mM magnesium chloride (Sigma, St. Louis, Missouri) and 30 µM (gamma-32P) ATP (DuPont). For all the enzymes either histone type HL (Worthington) or myelin basic protein is used as substrate. The assay is started by addition of protein kinase C enzyme incubated at 30°C for 10 minutes and stopped by adding 0.5 mL of cold trichloroacetic acid (Amresco) followed by 100 µL of 1 mg/mL bovine serum albumin (Sigma, St. Louis, Missouri). The precipitate is collected by vacuum filtration on glass fiber filters employing a TOMTEC™ filtration system and quantified by counting in a beta scintillation counter.

The compounds of the invention inhibit protein kinase C with an IC_{50} value of below 100 μm. In addition, the compounds of the invention selectively inhibit the beta-1 and beta-2 protein kinase C isozymes and have an IC_{50} value with respect to these isozymes of below 10 μm.

As an inhibitor of protein kinase C, the compounds are useful in the treatment of conditions in which protein kinase C has demonstrated a role in the pathology. Conditions recognized in the art include: diabetes mellitus and its complications, ischemia, inflammation, central nervous system disorders, cardiovascular disease, Alzheimer's disease, dermatological disease and cancer.

Protein kinase C inhibitors have been shown to block inflammatory responses such as neutrophil oxidative burst, CD3 down-regulation in T-lymphocytes, and phorbol-induced paw edema. Twoemy, B. et al. Biochem. Biophys. Res. Commun. 171: 1087-1092 (1990); Mulqueen, M.J. et al. Agents Actions 37: 85-89 (1992). Accordingly, as inhibitors of PKC, the present compounds are useful in treating inflammation.

Protein kinase C activity plays a central role in the functioning of the central nervous system. Huang, K.P. Trends Neurosci. 12: 425-432 (1989). In addition, protein kinase C inhibitors have been shown to prevent the damage seen in focal and central ischemic brain injury and brain edema. Hara, H. et al. J. Cereb. Blood Flow Metab. 10: 646-653 (1990); Shibata, S. et al. Brain Res. 594: 290-294 (1992). Recently, protein kinase C has been determined to be implicated in Alzheimer's disease. Shimohama, S. et al., Neurology 43: 1407-1413 (1993). Accordingly, the compounds of the present invention are useful in treating Alzheimer's disease and ischemic brain injury.

Protein kinase C activity has long been associated with cell growth, tumor promotion and cancer. Rotenberg, S.A. and Weinstein, I.B. Biochem. Mol. Aspects Sel. Cancer 1: 25-73 (1991). Ahmad et al., Molecular Pharmacology: 43 858-862 (1993). It is known that inhibitors of protein kinase C inhibitors are effective in preventing tumor growth in

animals. Meyer, T. et al. Int. J. Cancer 43: 851-856 (1989); Akinagaka, S. et al. Cancer Res. 51: 4888-4892 (1991). The compounds of the present invention also act as multidrug reversal (MDR) agents making them effective compounds when administered in conjunction with other chemotherapeutic agents.

Protein kinase C activity also plays an important role in cardiovascular disease. Increased protein kinase C activity in the vasculature has been shown to cause increased vasoconstriction and hypertension. A known protein kinase C inhibitor prevented this increase. Bilder, G.E. et al. J. Pharmacol. Exp. Ther. 252: 526-530 (1990). Because protein kinase C inhibitors demonstrate inhibition of the neutrophil oxidative burst, protein kinase C inhibitors are also useful in treating cardiovascular ischemia and improving cardiac function following ischemia. Muid, R.E. et al. FEBS Lett. 293: 169-172 (1990); Sonoki, H. et al. Kokyu-To Junkan 37: 669-674 (1989).

The role of protein kinase C in platelet function has also been investigated and as shown elevated protein kinase C levels being correlated with increased response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl. 1) 97A (1993). PKC has been implicated in the biochemical pathway in the platelet-activity factor modulation of microvascular permeability. Kobayashi et al., Amer. Phys. Soc. H1214-H1220 (1994). Potent protein kinase C inhibitors have been demonstrated to affect agonist-induced aggregation in platelets. Toullec, D. et al. J. Biol. Chem. 266: 15771-15781 (1991). Protein kinase C inhibitors also block agonist-induced smooth muscle cell proliferation. Matsumoto, H. and Sasaki, Y. Biochem. Biophys. Res. Commun. 158: 105-109 (1989). Therefore, the present compounds are useful in treating cardiovascular disease, atherosclerosis and restenosis.

Abnormal activity of protein kinase C has also been linked to dermatological disorders such as psoriasis. Horn, F. et al. J. Invest. Dermatol. 88: 220-222 (1987); Raynaud,

F. and Evain-Brion, D. Br. J. Dermatol. 124: 542-546 (1991). Psoriasis is characterized by abnormal proliferation of keratinocytes. Known protein kinase C inhibitors have been shown to inhibit keratinocyte proliferation in a manner that parallels their potency as PKC inhibitors. Hegemann, L. et al. Arch. Dermatol. Res. 283: 456-460 (1991); Bollag, W.B. et al. J. Invest. Dermatol. 100: 240-246 (1993). Accordingly, the compounds as inhibitors of PKC are useful in treating psoriasis.

Protein kinase C has been linked to several different aspects of diabetes. Excessive activity of protein kinase C has been linked to insulin signaling defects and therefore to the insulin resistance seen in Type II diabetes. Karasik, A. et al. J. Biol. Chem. 265: 10226-10231 (1990); Chen, K.S. et al. Trans. Assoc. Am. Physicians 104: 206-212 (1991); Chin, J.E. et al. J. Biol. Chem. 268: 6338-6347 (1993). In addition, studies have demonstrated a marked increase in protein kinase C activity in tissues known to be susceptible to diabetic complications when exposed to hyperglycemic conditions. Lee, T.-S. et al. J. Clin. Invest. 83: 90-94 (1989); Lee, T.-S. et al. Proc. Natl. Acad. Sci. USA 86: 5141-5145 (1989); Craven, P.A. and DeRubertis, F.R. J. Clin. Invest. 83: 1667-1675 (1989); Wolf, B.A. et al. J. Clin. Invest. 87: 31-38 (1991); Tesfamariam, B. et al. J. Clin. Invest. 87: 1643-1648 (1991).

The compounds of the invention are also isozyme-selective. The compounds preferentially inhibit protein kinase C beta-1 and beta-2 isozyme over the protein kinase C isozymes, i.e., alpha, gamma, delta, epsilon, zeta, and eta. In general, the compounds demonstrate a minimum of a ten fold differential in the dosage required to inhibit PKC beta-1 or beta-2 isozyme and the dosage required for equal inhibition of the alpha protein kinase C isozyme as measured in the PKC assay. Accordingly, compounds of the present invention inhibit beta-1 and beta-2 isozymes of protein kinase C at much lower concentrations with minimal inhibition of the other PKC isozymes.

Because of this selectivity, the compounds are particularly useful in treating those disease states in which protein kinase C isozyme beta-1 or beta-2 are associated. For example, the elevated blood glucose levels found in diabetes leads to an isozyme-specific elevation of the beta-2 isozyme in vascular tissues. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). A diabetes-linked elevation of the beta isozyme in human platelets has been correlated with their altered response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl 1) 97A (1993). The human vitamin D receptor has been shown to be selectively phosphorylated by protein kinase C beta. This phosphorylation has been linked to alterations in the functioning of the receptor. Hsieh et al., Proc. Natl. Acad. Sci. USA 88: 9315-9319 (1991); Hsieh et al., J. Biol. Chem. 268: 15118-15126 (1993). In addition, recent work has shown that the beta-2 isozyme is responsible for erythroleukemia cell proliferation while the alpha isozyme is involved in megakaryocyte differentiation in these same cells. Murray et al., J. Biol. Chem. 268: 15847-15853 (1993).

The compounds of Formula I are preferably formulated prior to administration. Therefore, yet another embodiment of the present invention is a pharmaceutical formulation comprising a compound of Formula I and one or more pharmaceutically acceptable carriers, diluents or excipients.

The present pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs,

suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 to about 500 mg, more usually about 5 to about 300 mg, of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the condition to be treated, the choice of compound to be administered and the chosen route of administration, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, the compounds of the present invention may be administered topically. Topical formulations are ointments, creams, and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon formation of the emulsion, the active ingredient (compound) customarily is added to an amount to achieve the desired concentration.

Gels comprise a base selection from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound incorporated into a topical formulation is not critical; the concentration should only be a range sufficient to permit ready application of the formulation to the an affected tissue area in an amount which will deliver the desired amount of compound.

The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to the effected

tissue in an amount affording from about 1 to about 500 µg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu g/cm^2$, more preferably, from about 50 to about 200 $\mu g/cm^2$, and, most preferably, from about 60 to about 100 $\mu g/cm^2$.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active agent	250
starch, dried	200
magnesium stearate	10
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active agent	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

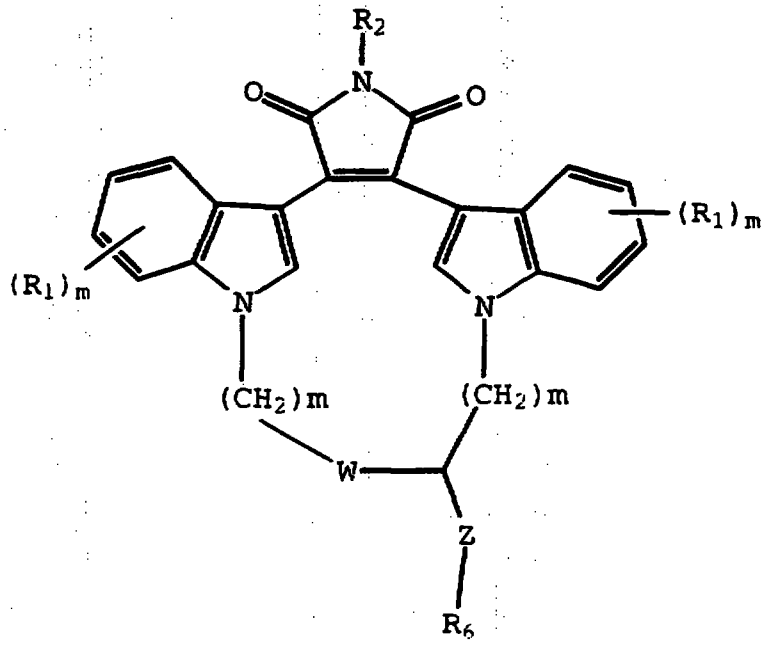
An aerosol solution is prepared containing the following components:

	Quantity (mg/capsule)
Active agent	0.25
ethanol	29.75
Propellant 22 (chlorodifluoromethane)	70.00
Total	100.00

The active compound is mixed with ethanol. The mixture is added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

We claim:

1. A compound of formula:



wherein:

W is O, -S-, or NH;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)$_2$, or -NHCO(C_1-C_4 alkyl);

R_2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

Z is -$(CH_2)_p$- or -$(CH_2)_p$-O-$(CH_2)_p$-;

R_6 is -NH(CF_3) or -N(CF_3)(CH_3);

m is independently 0, 1, 2, or 3; and

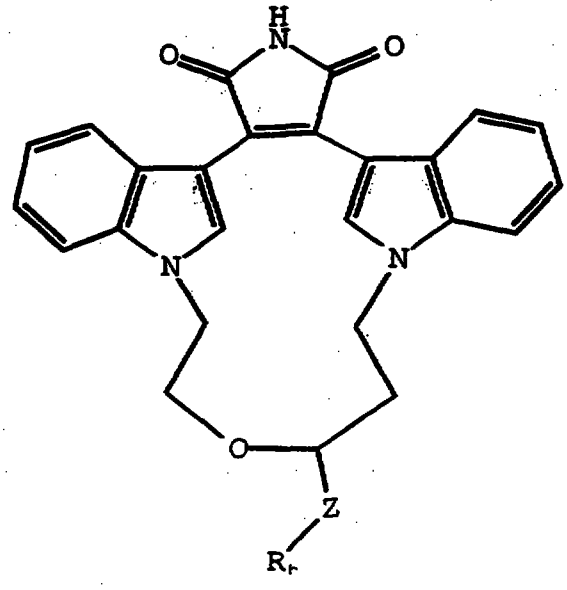
p is independently 0, 1 or 2; or

a pharmaceutically acceptable salt or solvate thereof.

2. A compound of Claim 1 wherein R_2 is hydrogen.

3. A compound of Claim 2 wherein W is a -O-.

4. A compound of Claim 3, which is of the formula:



wherein Z is $-CH_2-$; R_6 is $-NH(CF_3)$ or $N(CF_3)(CH_3)$.

5. A method for treating diabetes mellitus and its complications, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

6. A method of inhibiting protein kinase C, which comprises administering to a mammal in need such treatment a pharmaceutically effective amount of a compound of any one of Claim 1 through 4.

7. A method for treating central ischemia brain injury, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claim 1 through 4.

8. A method for treating cardiovascular ischemia, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

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9. A method for treating inflammation, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

10. A method for treating restenosis, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

11. A method for treating atherosclerosis, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

12. A method for treating psoriasis, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

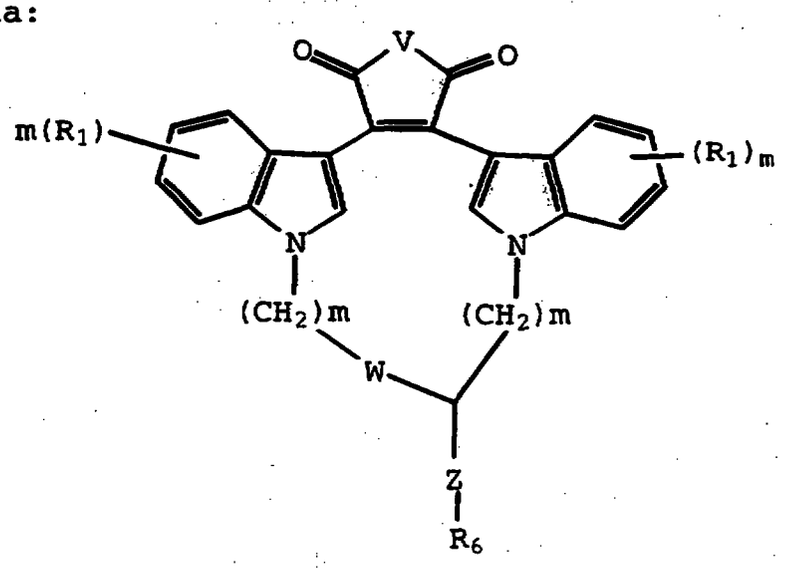
13. A method for treating Alzheimer's disease, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

14. A method for treating cancer, which comprises administering to a mammal in need of such treatment a therapeutic amount of a compound of any one of Claims 1 through 4.

15. A pharmaceutical formulations comprising a compound of any one of Claims 1 through 4 together with one or more pharmaceutically acceptable excipients, carriers, or diluents.

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16. A process of preparing a compound of any one of Claims 1 through 4, which comprises reacting a compound of the formula:



wherein:

V is -O- or N-CH_3;

W is O, -S-, or -NH;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)$_2$, or -NHCO(C_1-C_4 alkyl);

Z is -$(CH_2)_p$- or -$(CH_2)_p$-O-$(CH_2)_p$-;

R_6 is -NH(CF_3) or -N(CF_3)(CH_3);

m is independently 0, 1, 2, or 3; and

p is independently 0, 1 or 2.

with an excess of a mixture of a reagent and a C_1-C_4 alcohol in an polar aprotic solvent, said reagent being selected from hexamethyldisilazane or an ammonium salt.

17. A compound of any one of Claims 1 through 4 for use as a pharmaceutical.